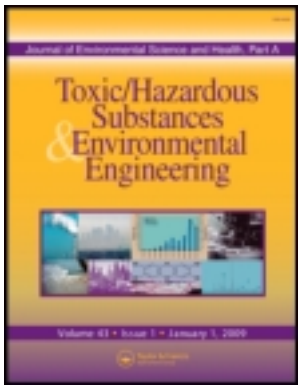

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Effects of salinity on the microbial removal of nitrate under varying nitrogen inputs within the marshland upwelling system

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The marshland upwelling system (MUS) utilizes the natural properties of wetland soils to treat domestic wastewater injected into the marsh subsurface as the wastewater moves upwards and outwards from the injection site. The system is different from coarse media based wetland treatment systems common in Europe, though it relies on the same principles. A laboratory study was designed to simulate field conditions in order to investigate and quantify the removal of nitrogen from the wastewater by pumping wastewater into the bottom of cores and observing the changes as the wastewater moved upward to the surface. Two nitrogen treatments (100 mg NH_4-N L^{-1} and 80 mg NH_4-N L^{-1}/20 mg NO_3-N L^{-1}) and two salinities (2 and 20‰) for each N treatment were studied. Dissolved organic carbon (DOC) demonstrated a removal efficiency of 90%, while NO_3-N had a removal efficiency of > 99% throughout the 84 days of the study. Higher salinity had a temporary, significant lower removal of DOC, while nitrate removal was high and consistent over time. Microbial biomass C (MBC) and denitrification enzyme activity (DEA) were measured to determine the role of microbial processes within the MUS. Wastewater introduction increased microbial growth at the column surface, which led to increases in denitrification/nitrification coupling and net N loss, as estimated by DEA. Salinity and organic matter were found to have significant negative and positive impacts, respectively, on DEA rates and MBC. An understanding of the impacts of salinity on specific microbially-mediated N transformations is critical for improving the efficiency of the MUS in coastal environments to determine the long-term sustainability.

Keywords: Treatment wetland, nitrogen, carbon, ammonia, nitrate, denitrification, denitrification enzyme activity (DEA), microbial biomass carbon, dissolved organic carbon, wastewater, salinity.

Introduction

A novel wastewater treatment system being tested in coastal wetland environments is the marshland upwelling system (MUS), which capitalizes, in part, on microbial transformations in soil. The MUS was developed as an alternative wastewater treatment system to address issues that are present in many coastal environments including Louisiana, such as high water tables, poor hydraulic soil conductivity, anaerobic soils, and saline groundwater. [1–3] The MUS differs from traditional constructed treatment wetlands, such as horizontal subsurface flow, surface flow, and vertical flow wetlands.

In horizontal subsurface flow wetlands, the water flows horizontally from inlet to outlet through the subsurface of the wetland. Surface flow wetlands most closely resemble natural wetlands and treat the wastewater as it moves through areas of open water and floating and emergent vegetation. In vertical flow wetlands, water is dispersed across the surface of the wetland and then treated as it moves vertically down through the sediment.[4] However, both the vertical subsurface flow and horizontal flow wetlands use an artificial bed media over short distances, while the MUS has a longer path length and vertical upward flow and utilizes soils comprised primarily of sand and silt.

The MUS (Fig. 1) utilizes natural wetlands in coastal environments and relies on the native soil and microbial population at *in situ* salinity for treatment. The system operates by collecting black and gray wastewater from coastal dwellings in a collection/distribution tank. Solids are allowed to settle out and the wastewater is then injected intermittently into the marsh subsurface. The intermittent injection cycles creative active injection and resting cycles allowing back pressure in the injection well to dissipate during resting cycles. The wastewater is radially dispersed from the injection point deep in the soil and moves upward

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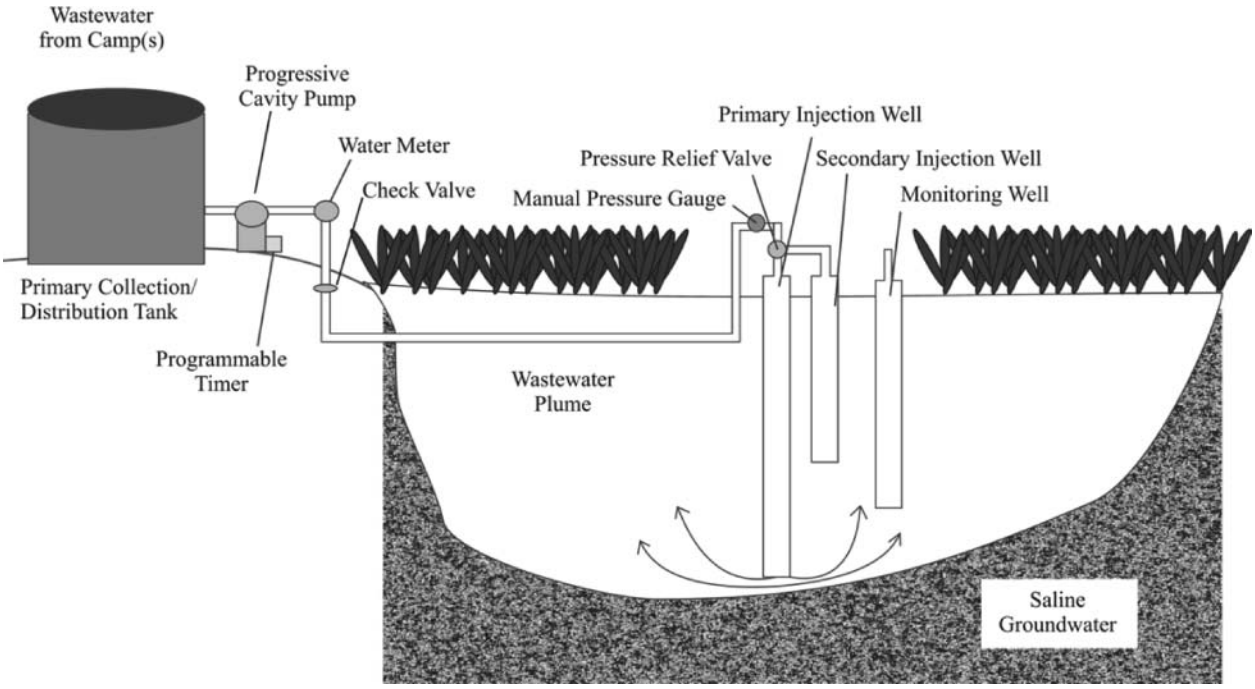

Fig. 1. Generic schematic of the marshland upwelling system.

and outward by natural groundwater flow and buoyancy forces due to the salinity differences between the fresher wastewater and naturally brackish or salt groundwater. As the wastewater moves through various oxidation-reduction zones within the subsurface, it is treated by a number of natural processes, including physical filtration, nutrient sorption by mineral and organic soil solids, plant uptake, and microbial decomposition and transformation.[5]

Wetland treatment systems rely heavily on microbial communities to treat nitrogen as microbes are able to permanently remove nitrogen as N_2 from the system.[6] Any environmental changes that affect the microbial community can affect the treatment of nitrogen. Higher salinities can have negative impacts on the microbial community.[7] As sea level rises, the salinity of coastal marshes in Louisiana will likely also rise, making the effect of salinity on microbially-mediated N processes important for the long-term use of the MUS.

Previous field studies have demonstrated effective short-term treatment of wastewater nitrogen (N) treatment in both salt and intermediate marshes.[2,8] However, these studies provided no information on the effect of specific soil properties on N treatment, attempted to determine microbial processes, nor investigated differences in treatment related to salinity. Consequently, a more detailed investigation of the specific processes occurring was undertaken to provide information that could potentially improve system performance and longevity. Thus, the objectives for this study were to determine: 1) the influence of soil properties on N treatment, 2) the microbial processes related to N treatment occurring within the MUS, and 3) potential influences of salinity on microbially-mediated processes.

Materials and methods

Setup

Nitrogen transformations within the MUS were investigated using a laboratory column experiment, as field conditions are essentially unbounded, thus preventing a quantitative and detailed process-level investigation without significant cost and manpower. Three experimental treatments (salinity, nitrogen, and plants), with two levels each were chosen to evaluate the efficiency of MUS N removal (Table 1).

The N levels (80 mg NH_4-N L^{-1}/20 mg NO_3-N L^{-1} and 100 mg NH_4-N L^{-1}) were chosen as representative of levels observed at field sites and in a pilot study.[2,8,9] Each salinity treatment also had a planted control that received no wastewater to compare wastewater treated columns to control columns receiving only the corresponding saltwater. However, the majority of the plants died partway through the study and, therefore, the potential influence of plants on nitrogen treatment is not discussed. Treatment combinations were duplicated and randomly placed for a total of 16 wastewater-treated columns and four control columns. For a more detailed description of column construction, see Putnam (2009).[9]

Standard synthetic wastewater[10] was used with the following modifications: salt, nitrogen, and phosphorus levels

Table 1. Layout of experimental design showing three treatments used. Each treatment combination was duplicated for a total of 20 experimental units (or columns).

Treatments		
Nitrogen	*Salinity*	*Plants*
80 mg NH_4-N L^{-1}	2‰	Planted
		Unplanted
20 mg NO_3-N L^{-1}	20‰	Planted
		Unplanted
100 mg NH_4-N L^{-1}	2‰	Planted
		Unplanted
	20‰	Planted
		Unplanted
No wastewater	2‰	Planted
	20‰	Planted

A part per thousand is abbreviated as ‰.

were increased to the desired wastewater concentrations, kaolin (a mineral) was not added, a supplement (Reef Plus, Aquatic Ecosystems) containing trace nutrients was added, and the amount of beer (carbon source) was decreased from 6% to 1%. The amount of beer was decreased as preliminary tests found the dissolved organic carbon (DOC) level to be high in comparison to field wastewater levels. Wastewater-treated columns received 12 mg PO_4-P L^{-1}, 200 mg C L^{-1}, and either 80 mg NH_4-N L^{-1}/20 mg NO_3-N L^{-1} or 100 mg NH_4-N L^{-1}, based on field and lab studies.[2,8,9] Saltwater solutions were made using individual components.[11]

Wastewater was added at an injection flow rate of 0.03 L min^{-1} at a frequency of 1 hour every 2 days. The injected wastewater was sampled at every addition on a two day cycle. Samples were collected from each sampling port (Fig. 2) before wastewater addition on days 0, 5, 14, and every 14 days thereafter for a total of 84 days. Salinity, pH, redox potential, and temperature of the samples were measured at time of collection. Redox potential was measured with a SCE reference electrode and corrected to a standard hydrogen reference electrode and expressed as E_h.[12] At the end of the study, columns were sliced into seven sections as shown in Figure 2. Each section was then homogenized and stored at 4°C until further analysis.

Soil and water analyses

Sand, silt, and clay fractions were measured using sieve (ASTM C117, C136) and hydrometer analyses (ASTM D422).[13] Pre-study, soils were analyzed for organic matter (OM) content and total carbon and nitrogen (TC and TN). Post-study, soils were analyzed for organic matter (OM) content and total carbon and nitrogen (TC and TN). Organic matter was measured as loss on ignition at 435°C.[14] Total C and TN were determined on dried, ground samples using a Costech Elemental Combustion System (Valencia, CA).

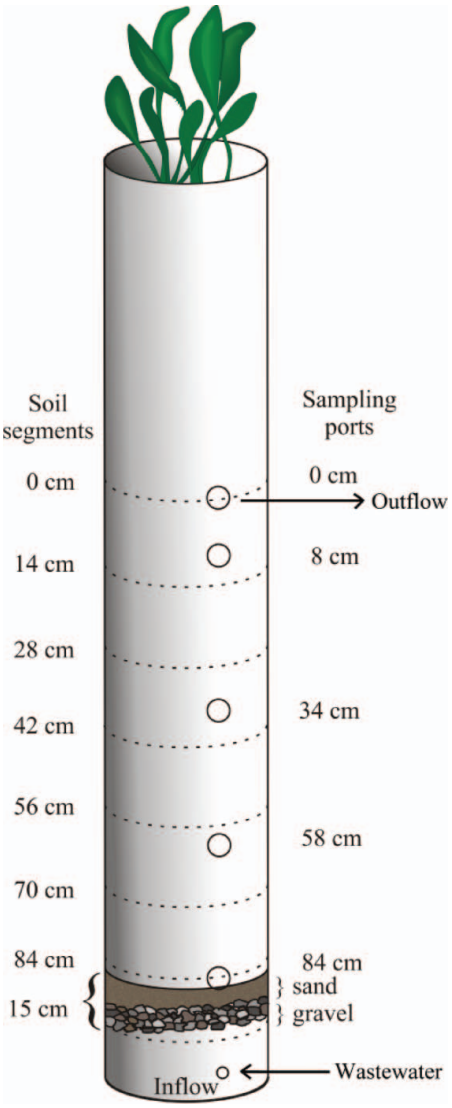

Fig. 2. Schematic of column setup. Sampling port depths are indicated on right. Soil segments cut from column at end of experiment are indicated on left. The distribution plate was installed beneath gravel (color figure available online).

The water samples were filtered (0.45 μm) and measured for NO_3-N + NO_2-N (NO_X, EPA method 353.2) and DOC. All water samples were preserved at pH < 2 and stored at 4°C until analysis, except for NO_X, which was kept at 4°C and analyzed within 48 h of sampling. Nitrate + nitrite was measured on a Seal Analytical AQ2 Automated Discrete Analyzer (Mequon, WI). Dissolved organic carbon was measured directly after acidification with HCl (pH < 2) using a Shimadzu TOC-V_{CSN} analyzer (Columbia, MD).

Soil sections were analyzed for microbial biomass carbon (MBC) and denitrification enzyme activity (DEA). Extractants for MBC were filtered using 0.45 μm membrane filters and stored at 4°C until analysis. Microbial biomass C was measured using ~ 2 g moist soil following the chloroform fumigation method after Vance et al.[15]

Table 2. Percent sand, silt, and clay and initial mean organic matter content, cation exchange capacity (CEC), total carbon, and total nitrogen for soil used in columns.

	cm below surface		
	0 - 15		*15 – 84*
Property	20‰	2‰	All
Sand (%)	13	22	78
Silt (%)	72	58	18
Clay (%)	15	20	4
USDA classification	silt loam	silt loam	loamy sand
Organic matter (%)	24	77	1.3
CEC (centimoles of cation charge kg^{-1} soil)	-	-	14.5
Total carbon (g C kg^{-1} soil)	-	-	10
Total nitrogen (g N kg^{-1} soil)	-	-	BDL[†]

[†]Below detection limit of 0.5 g N kg^{-1} soil.

Table 3. Overall mean of influent parameters.

	Treatment		
Parameter	*A*	*N*	*C*
DO (mg L^{-1})	7.50 ± 0.44 (54)	7.52 ± 0.40 (54)	7.69 ± 0.40 (50)
pH	7.85 ± 0.41 (86)	7.88 ± 0.36 (86)	7.82 ± 0.19 (32)
DOC (mg C L^{-1})	224 ± 57 (72)	216 ± 57 (72)	42 ± 32 (26)
NH_4^+ (mg N L^{-1})	94 ± 7.9 (86)	75 ± 7.4 (86)	0.61 ± 0.72 (31)
NO_X (mg N L^{-1})	0.12 ± 0.25 (86)	18 ± 2.2 (86)	0.06 ± 0.08 (32)

n = ().
Treatments are abbreviated as 100 mg NH_4-N L^{-1} (A) vs. 80 NH_4-N L^{-1}/20 mg NO_3-N L^{-1} (N), and control (planted columns receiving no wastewater, C).

and White and Reddy[16] and measured directly using a Shimadzu TOC-V_{CSN} analyzer (Columbia, MD). Denitrification enzyme activity was measured using ~ 5 g moist soil in glass serum bottles evacuated with 99.99% O_2-free N_2 gas using the acetylene block technique after White and Reddy.[6] The N_2O for the DEA analysis was determined using a Shimadzu GC-8A equipped with an ECD detector (Columbia, MD) equipped with a 1.8 m × 2 mm i.d. stainless steel column with Poropak Q (0.177 – 0.149 mm; 80 – 100 mesh; Supelco, St. Louis, MO).

Data analysis

SAS® software[17] and SigmaPlot® software[18] were used to analyze the data. All statistical tests were performed at a significance level of $\alpha = 0.05$. A two-way ANOVA (salinity and nitrogen-form) with a split-plot addition for depth was applied to compare treatments. Controls were excluded from the two-way ANOVA as they led to an unbalanced design. In order to compare controls to wastewater treated columns, comparisons to control data were made using a one-way ANOVA (using the 10 treatment combinations) with linear combinations between controls and wastewater treated columns. Soil characteristics were related using Pearson's product correlation analysis. Denitrification enzyme activity (DEA) values from 0 – 28 cm were log transformed to meet normality requirements of the statistical tests.

Results and discussion

Overall soil and water characteristics

The soil added to the columns was a silt loam from 0 – 15 cm and a loamy sand from 15 – 84 cm (Table 2)[19] to mimic field conditions. For all soil and microbial data measured, means were significantly higher at the surface and decreased with depth. There were no significant differences seen in the soil sections from 28 – 84 cm (below the surface) and the bottom sand section. As such, non-significant data was pooled together for simplification of graphical and tabular presentation. The top 15 cm of soil in each column contained organic soil obtained from a marsh of a similar salinity to simulate field conditions. Thus, from 0–14 cm, the soil was nearly entirely comprised of high organic material and, from 14–28 cm, the soil was a mixture of the surface OM layer and the soil found from 28–84 cm. This difference accounted for the mid-range values found in the 14–28 cm for most parameters.

Mean influent parameters were consistent throughout the study (Table 3) as well as the mean salinity and pH of the effluent and the redox potential of the soil. Mean wastewater column measures for temperature, pH, and redox potential were 22.4 (± 1.0)°C, 6.83 (± 0.36), and −87 (± 117) mV, respectively. Salinity in the columns averaged 23.0 (± 3.8) and 3.1 (± 3.4)‰ for the 20 and 2 treatments, respectively.

Organic matter content at the end of the study ranged from 1.6–62%, with the lowest percentages found in the subsurface (28–84 cm). Total C ranged from 5–301 g C kg^{-1} soil (Table 2). Total C was significantly higher ($p < 0.001$) in wastewater treated columns versus control columns, an indication that addition of wastewater increased TC levels. Total N was only detectable within the 0–28 cm soil interval and ranged from 0.89–22 g N kg^{-1} soil, while it was below the detection limit of 0.5 g N kg^{-1} soil (Table 2) at all other depths.

Carbon treatment

Dissolved organic carbon in the wastewater was significantly reduced ($p < 0.001$) from an influent mean of 220

to an overall effluent mean of 22.7 (± 16.8) mg C L^{-1}. Among columns receiving wastewater, the higher salinity columns had a significantly higher DOC ($p < 0.05$) from 0 to 8 cm relative to the lower salinity treatment at this depth. This difference was likely due to increased microbial activity in the low salinity columns. Microbial biomass C had a higher mean, at the surface, in the low salinity columns than in the high salinity columns (9.91 vs. 4.22 mg C kg^{-1} soil, respectively). High salinities have been found to have a negative impact on microbial activity.[20–23] The significant differences seen in DOC levels in the effluent were only short-lived, as these differences dissipated by day 56, suggesting that the microbial population may acclimate to the higher salinity over time.

Microbial biomass C ranged from 0.02–23.4 g C kg^{-1} soil. Overall, the highest levels of MBC were found in the top 0–14 cm (Fig. 3) and were significantly higher ($p < 0.001$) in wastewater treated columns than control columns at this depth. Though MBC is not always directly related to microbial activity (due to inactive or resting microbial groups), it gives an indication of the total microbial pool present. Thus, wastewater increased MBC and, likely, microbial activity, due to the additional DOC load.

The higher mean of MBC found near the surface indicated there was a higher microbial activity at the surface (more oxidized, more C) and a lower activity at deeper depths where the environment was more reduced and lower in C. Microbial activity and OM decomposition in reducing environments are generally much slower than in oxygenated environments.[24] D'Angelo and Reddy[25] found that carbon mineralization was three times slower in anaerobic wetland environments, while White and Reddy[26] found that nitrogen mineralization was six times slower.

Nitrate treatment

Nitrate N was significantly ($p < 0.001$) reduced from an influent mean of 18.43 (± 2.18) mg NO_X-N L^{-1} to an overall effluent mean of 0.06 (± 0.15) mg NO_X-N L^{-1} in columns receiving wastewater. (Nitrite was measured separately and was not a significant fraction of NOx, < 1%.) To compare effects among the nitrate treatment, statistical analyses were run only on columns receiving nitrate over the entire course of the study. Among these columns, nitrate levels were significantly higher ($p < 0.05$) at the surface relative to the subsurface (−8 to −84 cm), but only among columns of the lower salinity. This suggests that the high levels of NH_4^+ in the wastewater were likely converted to NO_3^- via nitrification at the more oxidized surface, and that nitrification was inhibited at the higher salinity.

There was no significant difference in NO_X between nitrogen treatments. Wastewater nitrate entered the columns from the bottom and was consumed within a short distance.

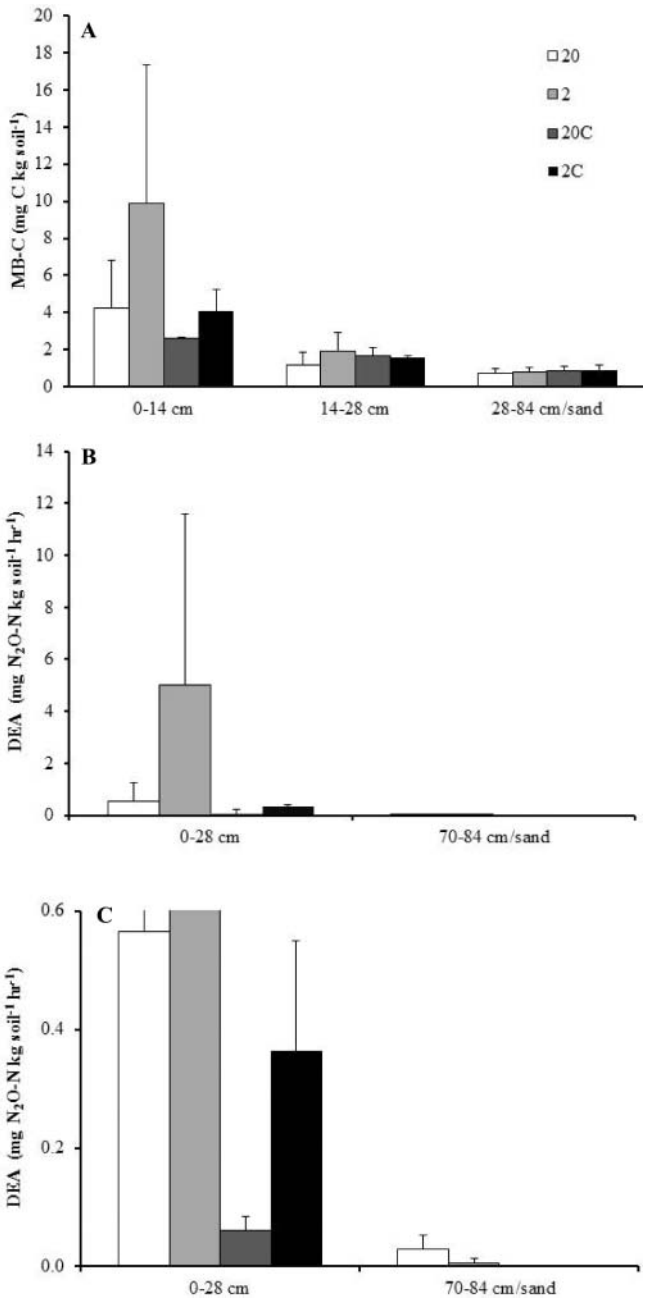

Fig. 3. Mean microbial biomass carbon (MBC, graph A) and denitrification enzyme activity (DEA, graphs B and C) of columns by depth below surface. Data for salinity treatments 2 and 20‰ is presented for control (planted columns receiving no wastewater) and wastewater treated columns. Data not shown for DEA is below detection limit (0.001 mg N_2O-N kg^{-1} soil h^{-1}). Graph B shows DEA of all treatments. Graph C shows the same information at a smaller scale. Error bars show variability of each treatment combination. C = control, no wastewater added; 2 = 2‰; 20 = 20‰.

This is evidenced from samples at the first (lowest) sampling port (−84 cm) showing no difference in the NO_X concentration among columns receiving NO_3^- versus all columns not receiving NO_3^- (Fig. 4). The reduction of NO_3^- was

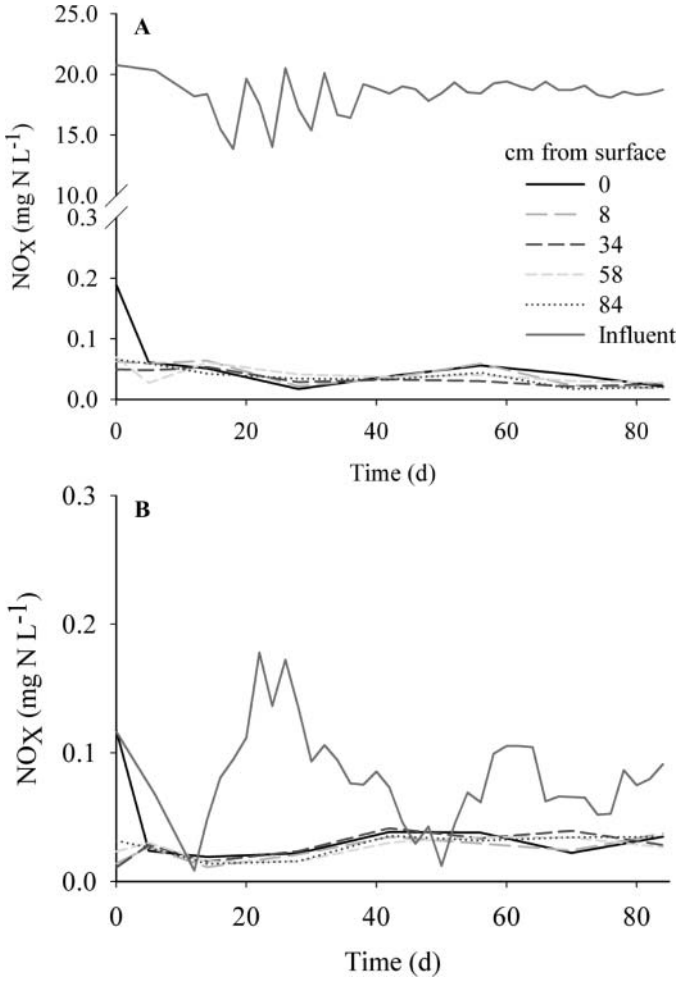

Fig. 4. Representative graphs of NO_X versus time. Lines represent the mean of NO_X at each depth. Graph A shows 20‰, planted, 80 NH_4-N L^{-1}/20 mg NO_3-N L^{-1} treatment combination and Graph B shows 2‰, planted, 100 mg NH_4-N L^{-1}.

likely due to denitrification, as measured by DEA. The mean surface (0 to −8 cm) redox potential was 37 and the mean subsurface (−8 to −84 cm) redox potential was −118 mV. Though the average redox potential for initiation of denitrification typically occurs from 100–300 mV,[27] denitrification can occur at a wide range of redox potentials, as the process is largely dependent upon the availability of NO_3^- and C.

Understanding the factors influencing denitrifiers will help to improve the denitrification potential in these systems as denitrifiers convert NO_3^- to N_2, thus removing nitrogen from the system. Denitrification enzyme activity (DEA) provides a measure of the rate at which denitrification is occurring within wetland systems.[6,28] Because of the low number of data points above the detection limit for DEA, data were placed into two groups: 0–28 cm, and 70–84 cm/sand layer. The majority of DEA occurred near the surface between 0–28 cm where DEA ranged from 0.01–22.6 mg N_2O-N kg^{-1} h^{-1} and was significantly greater ($p < 0.001$) in wastewater treated columns than control columns (Fig. 3). In the bottom 70 – 84 cm/sand layer, DEA was below 0.06 mg N_2O-N kg^{-1} h^{-1} for all treatments likely related to the very low C content of this layer.[25]

If denitrification was the primary microbial process occurring, the treatment receiving NO_3^- would be expected to have a significantly higher DEA in the soil near the wastewater entrance (70–84 cm) than the treatment that did not receive NO_3^-. There was no significant difference between the treatment receiving NO_3^- and the treatment receiving none due to high variability in the DEA values. However, all of the DEA detected from 70–84 cm occurred in the treatment receiving NO_3^-. In contrast, the percentage of DEA occurring at the surface (0 – 28 cm) was nearly equal between the two treatments. Here, 53% of the DEA that occurred was in the treatment receiving no NO_3^-. The high rate of DEA occurring in this treatment can be explained by nitrification/denitrification coupling at the surface,[29] as the wastewater NH_4^+ is converted to NO_3^- by nitrifying bacteria. Nitrification/denitrification coupling at the surface is also supported by the NO_X data where columns receiving only NH_4^+ had NO_X in the effluent.

High levels of NH_4^+ were present in all columns and likely underwent little change in chemical form as the wastewater moved vertically up the column (through this anaerobic region) until the surface was reached. Although some NH_4^+ would become sorbed to the soil as it traveled, the high levels of NH_4^+ reaching the surface[9] and introduction of oxygen at the surface and rhizosphere would actively stimulate nitrification and, subsequently, denitrification, as NO_3^- diffused into anaerobic regions. Because of the close proximity of aerobic and anaerobic soil environments at the soil surface and within the rhizosphere, efficiency of nitrification and denitrification is increased.[29] Though nitrification rates were not measured, the detection of NO_X and DEA within the 100% NH_4^+ treatment strongly suggests NH_4^+ was being converted to NO_3^- providing the substrate for denitrification.

However, if the loading of NO_3^- is considered relative to the DEA measured within the subsurface, the nitrogen load was greater than the DEA would suggest. The level of NO_3^- loaded was 0.14 mg N kg^{-1} soil h^{-1}, which was five times higher than the mean DEA measured at that layer (0.026 mg N kg^{-1} soil h^{-1}). Though the rates of NO_3^- loading and DEA are determined using different methods (NO_3^- loading is an average, while DEA is a final measurement), the difference in rates is large enough to warrant further consideration.

The NO_3^- load was still considerably higher, even considering the highest DEA measured at this depth (0.057 mg N kg^{-1} soil h^{-1}). Though the DEA present in a soil is highly variable[30] and could account for the differences seen in this study, the possibility exists that anammox or dissimilatory nitrate reduction to ammonium (DNRA) occurred. Anammox occurs under strongly reducing

Table 4. Correlation matrix of soil properties as compared with microbial properties for column soils.

	Organic matter	*Microbial biomass C*	*Total C*
Microbial biomass C	0.85		
Total C	0.99	0.84	
DEA	0.66	0.39	0.69

DEA = denitrifying enzyme activity.
All r values listed are significant at $p < 0.01$.

conditions where NO_3^- and NH_4^+ are used in conjunction by microorganisms to produce N_2.[31] Anammox bacteria would directly compete for NO_3^- with denitrifiers, thus reducing the DEA that could occur, but still removing N from the system.

However, in DNRA, NO_3^- reduces to NH_4^+ as opposed to the N_2 gas formed during denitrification, which would be undesirable as N is not lost from the system through this process. Factors thought to favor DNRA include highly reduced soils (as it consumes eight electrons as opposed to the five electrons consumed during denitrification) and high organic carbon content (as DOC from the incoming wastewater).[31] Both the conditions necessary for anammox and DNRA occurred during the study, so it is likely one or both transformations may have occurred.

Denitrification decrease with increased distance from the surface is consistent with other studies and is correlated to a number of influences, particularly the availability of OM and NO_3^-.[6,32] The higher levels of TC, OM, and MBC present within the top 28 cm contributed directly to the higher levels of denitrification occurring, as the bottom 14 cm contained small amounts of these parameters.[33] The DEA was also positively correlated with TC ($p < 0.001$, $r = 0.69$) and MBC ($p < 0.01$, $r = 0.39$) (Table 4). The correlation between DEA and TC and MBC demonstrates the influence of carbon on denitrification. Carbon is necessary for denitrification as it supports requirements for both energy and cellular synthesis.[33, 34] Significant relationships between denitrification rates and water soluble organic carbon have been shown for a wide range of wetland soil types.[25]

The salinity interaction was significant from 0 – 28 cm ($p < 0.05$), where the DEA at the low salinity was greater than the high salinity (Fig. 3, data shown without log transformation). A higher salinity had a significant negative impact on DEA. Increased salinities are related to decreases in denitrification activity[7,35] and could explain, in part, why the low salinity treatment had a higher DEA rate from 0 – 28 cm.

MUS as wastewater treatment system

The overall mean removal efficiency for DOC using subsurface injection of synthetic wastewater was 90%. A study that looked at the treatment of municipal wastewater in a constructed mangrove swamp found a removal efficiency of 71%.[36] In a horizontal subsurface flow wetland treating synthetic wastewater, a removal efficiency of 93.2% was observed,[37] while in a different subsurface flow wetland treating municipal wastewater, a removal efficiency of between 68 and 72% was observed.[38] Generally, this study had a higher removal efficiency than other wetland wastewater treatment systems due to increased path length compared to more traditional vertical flow systems. An increased removal of DOC is important as C loading to surface waters can deplete the oxygen.

Removal efficiencies for NO_X were upwards of 99%, within just a few cm from the injection point at the base of the core during the entire length of the study. Previous field studies of the MUS also found no detectable amounts of NO_X in the marsh subsurface.[2,8] In comparison, a laboratory study treating synthetic wastewater saw nitrate removal efficiencies of 98% over the course of 20 months.[39] Two natural, forested, treatment wetlands receiving wastewater from municipal sources had 100% removal efficiency.[40] Planted surface flow wetlands, treating nitrate-contaminated groundwater, had efficiencies ranging from 70–99% as compared to unplanted surface flow wetlands, from the same study, that had a 55% removal efficiency.[41] Overall, this study, representing subsurface injection of wastewater, showed NO_X removal efficiencies similar to or higher than other traditional wetland treatment systems.

The range of DEA found in wetland systems is varied. Flite et al.[32] reported DEA levels ranging from 0–0.21 mg N_2O-N kg^{-1} soil h^{-1} in a riparian wetland, White and Reddy[6] from 0.004–7.75 in an Everglades Water Conservation Area, and Schipper and McGill[42] from 0.035–1.410 in soil irrigated with dairy processing effluent. Hunt et al.[43–45] has studied DEA in various different wetlands treating swine wastewater with DEA ranging from 0.210 – 0.516 mg N_2O-N kg^{-1} soil h^{-1},[44] a riparian zone adjacent to a swine wastewater spray field ranging from 0.003 – 1.66,[43] and a marsh-pond-marsh constructed wetland treating swine wastewater ranging from 0.06 – 1.13.[45] The measurable DEA in this studied ranged from 0.001 – 22.6 mg N_2O-N kg^{-1} soil h^{-1} and, if the two highest outliers (of 10.15 and 22.6) are excluded, the highest is then 6.39 mg N_2O-N kg^{-1} soil h^{-1}.

Excluding the two highest, the range of this data is comparable to other DEAs found in the literature. The highest DEA from this study is more than double any DEA found in other literature and was found in one of the columns containing a surficial microbial mat. It is possible the high productivity of microbial mats contributed to the high DEA found in this column and may be an area of research warranting investigation. However, differences in wastewater composition, bounded treatment area, and other environmental factors may also have contributed to differences in the DEA levels found in addition to any natural heterogeneity.[30]

The higher microbial activity (as measured by DEA) seen in this study under the lower salinity may be in part to the influence of salinity on microbial processes. Increases in salinity have been shown to lead to decreases in the microbial biomass and rate of denitrification.[7,20–22,46,47] Researchers have shown that agricultural practices resulting in increased salinity have led to a decrease in the size and activity of the soil microbial biomass.[47]

Seitzinger et al.[22] showed an indirect salinity effect on denitrification caused by a reduction in nitrification rates, which was due to reduced availability of extractable NH_4^+. Higher salinities are known to increase physiological stress in microbes, which could lead to a decrease in microbial diversity.[35,48,49] Yoshie et al.[35] has shown that salinities approaching that of seawater decrease the nitrite reductase gene (required for denitrification) diversity in wastewater treatment systems. Loss of genetic diversity can lead to reduced physiological diversity, robustness and resilience, and a greater susceptibility to environmental perturbations.

Although salinity may have a significant impact on N reduction, the properties of the soil are likely even more influential. Microbial activity, particularly DEA, was largely dependent upon the amount of carbon available. A majority of the soil characteristics measured were higher for the low salinity treatment, which was coincident with a higher OM and TC content, a higher MBC as well as increased rates of DEA. The initial OM content (from 0–14 cm depth) of the columns for the low salinity was 77%, whereas the high salinity surface soil was 24%. The higher OM content for the low salinity provides an explanation for why the low salinity treatment soil and microbial parameters were higher from 0–14 cm. Additionally, the influences of plant uptake could provide a significant increase to the treatment of nitrogen.

Although not included in the results of this paper, as the plants died partway through the study, plants can provide significant treatment of nitrogen by introducing oxygen into the rhizosphere and, thus, increasing the amount of ammonium converted to nitrate via nitrification and, subsequently nitrate to N_2 via denitrification.[50] Plants can also provide treatment of nitrogen through the direct uptake of the various nitrogen forms. However, this would only provide a temporary removal of nitrogen, because as the plants died they would be incorporated back into the marsh as organic-N.

Denitrification is the primary process which permanently removes nitrogen from the MUS, as wastewater is injected into the subsurface (i.e., anaerobic zone). The consumption of NO_3^- indicates that any conversion of NH_4^+ to NO_3^- before injection will result in treatment of nitrogen within the MUS as NO_3^- is consumed within anaerobic zones provided enough C is present in the wastewater. Alternatively, if oxygen could be introduced into various regions of the subsurface, this could potentially lead to an increase in nitrification and, therefore, denitrification in the nearby subsurface and a loss of nitrogen from the system.

However, the amount and frequency of oxygen introduction would need to be studied to determine the amount of oxygen needed to stimulate nitrification/denitrification coupling as denitrifiers are inhibited by oxygen.[51] Any NH_4^+ reaching the surface would be treated via nitrification/denitrification coupling through proximity to the surface or rhizosphere.[29] Available NH_4^+ would be nitrified to NO_3^- in aerobic regions and some NO_3^- would then diffuse into nearby anaerobic regions where it could be subsequently denitrified and lost from the system.

However, it is not desirable for untreated wastewater to reach the marsh surface, as this would defeat the purpose of the MUS, which is to treat wastewater before it reaches the surface and potentially contaminates surface water bodies. Thus, more work should be done on enhancing nitrification before wastewater injection. If nitrification can be achieved in the collection tank prior to injection, then NH_4^+ loading can be reduced with a concomitant increase in NO_3^- loading. This alteration will lead to a more direct removal of N through denitrification and potentially increase the longevity of the MUS with regards to N.

Conclusions

This study found DOC to be treated with a removal efficiency of 90% in the MUS. A higher salinity had a significant negative impact on the treatment of DOC in the short-term. However, over time, the treatment of DOC under the higher salinity columns reached the lower salinity columns suggesting the microbial population acclimated.[23] Nitrate was removed with $> 99\%$ efficiency. Introduction of wastewater significantly increased microbial activity and biomass at the surface of columns and, subsequently, led to increases in nitrification and denitrification rates, as estimated by DEA.

Of particular importance for the viability of this technology for N removal is the fact that if wastewater reaches the surface, N may contaminate the surrounding surface waters. Though coupled nitrification/denitrification would be an important mechanism for removal of N at the surface, denitrification in the subsurface would create the best scenario, as the likelihood of N contaminating surface waters would be greatly reduced. Thus, it would be beneficial for NH_4^+ to be converted to NO_3^- before injection into the subsurface, to increase the probability that denitrification will occur.

This study demonstrated that an increased understanding on the factors controlling the soil biogeochemical C and N cycling can lead to important strategies for improvements in this novel wastewater treatment technology. A better understanding of the linkages between the microbial community and ecosystem function can lead to improvements in the use of wetlands for wastewater treatment and for assessing the effects of perturbations on wetland ecosystems, such as environmental and pollutant variables.[52]

Therefore, consideration of the microbial transformations occurring and facilitating these transformations to improve wastewater treatment is vital to improve the long-term sustainability of the MUS in coastal wetland systems.

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